Chemerin: a Novel Adipokine in the Regulation of Angiogenesis

Claire J. Stocker[1], Mohamed S. Zaibi[1], Kiymet Bozaoglu[2], David Segal[2], Jeremy B.M. Jowett[3], Ken Walder[2], Jonathan R.S. Arch[1] , Michael A. Cawthorne[1]

[1]Clore Laboratory, University of Buckingham, Buckingham, UK, [2]MRU, Deakin University, Geelong, and [3]BakerIDI, Melbourne, Australia

Introduction

Obesity is commonly associated with type 2 diabetes, cancer, hypertension, dyslipidemia, and coronary heart disease. Protein hormones secreted by adipose tissue play a significant role in these disorders. Adipose tissue is a highly vascularised organ and consequently the expansion of adipose tissue that occurs during the development of obesity is dependent on the formation of new blood vessels, termed angiogenesis. Chemerin, a recently identified protein hormone (cytokine) secreted by adipose tissue, was shown to be associated with characteristics of the metabolic syndrome, including obesity, plasma triglycerides and blood pressure in plasma samples from several independent human cohorts. Chemerin may play an important role in the development of obesity and metabolic syndrome, but how this is achieved is still unknown.

Aims

Recent studies have shown that chemerin levels in the blood are influenced by genetic factors, but we also identified variations within genes that may influence chemerin levels and have found a link between chemerin and angiogenesis. Using functional studies we investigated whether chemerin participates in angiogenesis.

Methods

Capillary development was modelled *in vitro* using the AngioKit™ model which accurately reproduces the different phases of the angiogenesis process using a co-culture of early passage human endothelial cells with human interstitial cells. The treatments included control (C), DMSO (0.1%), VEGF (2 ng/ml), suramin (20 mM) (S) and recombinant human chemerin (0.1, 0.3, 1, 3 and 10 ng/ml (Ch) in the presence or absence of 25 mM PD98059 (PD), an inhibitor of MAPKK/MEK. Capillary development and tubule formation was followed by immunodetection using a specific endothelium detection marker CD31 (Platelet endothelial cell adhesion molecule). The quantitative measurement of total vessel number, total tubule length and number of junctions were scored using image analysis in triplicate experiments. Data are expressed as percentages relative to control wells. Effects of recombinant human chemerin, VEGF and PD98 were analysed by one-way ANOVA followed by Dunnett's or Bonferroni post hoc tests as appropriate.

Results are means ± SE. Statistical significance for effects is given as **P*<0.05; ***P*<0.01; ****P*<0.001 compared with the vehicle control.

Conclusion

Capillary development was modelled *in vitro*. Recombinant chemerin and VEGF increased total tubule length, branch and total microtubule number, in a concentration-dependent manner. The MEK1 inhibitor PD98059 significantly reduced chemerin-induced angiogenesis. Its effects are dependent on MAPKinase activity. Obesity is associated with adipose and vascular remodelling, an inadequate blood supply leading to hypoxia and systemic inflammation. Exogenous chemerin promotes the formation of blood vessels. Thus it may play a role in the development of obesity promoting angiogenesis within an expanding adipose tissue mass.

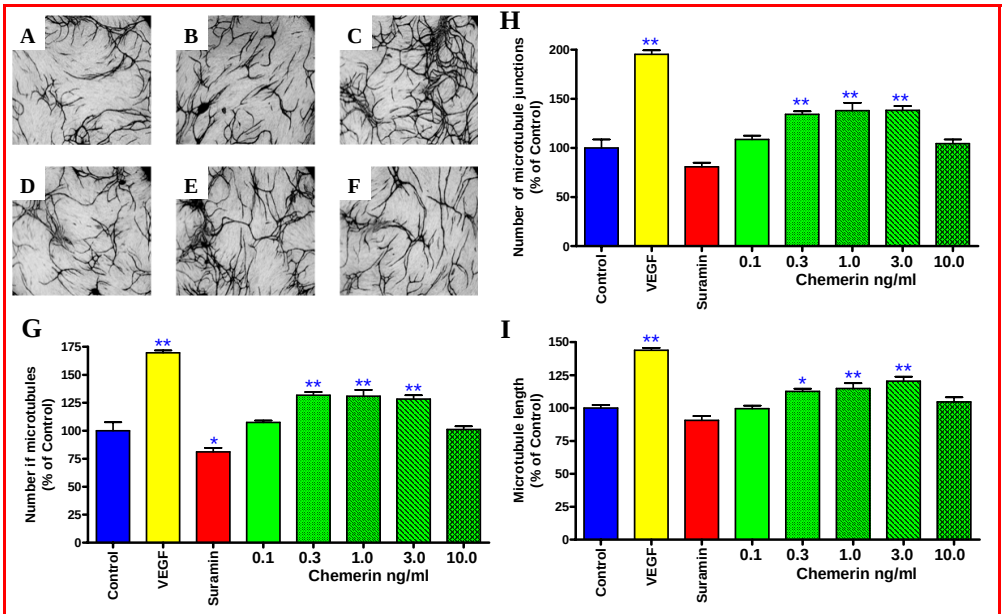

Figure 1. Effect of recombinant chemerin concentration on blood vessel formation by endothelial cells in a co-culture system. The co-culture of endothelial cells and fibroblasts was used to study the tube formation by endothelial cells. The AngioKit was seeded with cells on day 0, and the optimised growth medium was changed on days 1, 3, 5, and day 7. The cells were fixed and stained for CD31, on day 9. Suramin (20 µM) and VEGF (2 ng/ml) were used as negative and positive controls, respectively. Recombinant human chemerin was added to each well at 0.1, 0.3, 1, 3 and 10 ng/ml. (**A**) control: cell medium only; (**B**) inhibition of tubule formation by Suramin (20 µM); (**C**) VEGF (2 ng/ml), induced tubule formation as well as VEGF-induced increased branching of tubules and formation of *in-vitro* capillaries. (**D**), (**E**) and (**F**) represents images of the co-culture after 9 days incubation with 0.1, 1 and 10 ng/ml of recombinant human chemerin. Number of microtubules (**G**), tubule anastamoses (**H**) and microtubule length (**I**). Four images were captured per well, from four independent quadrants.

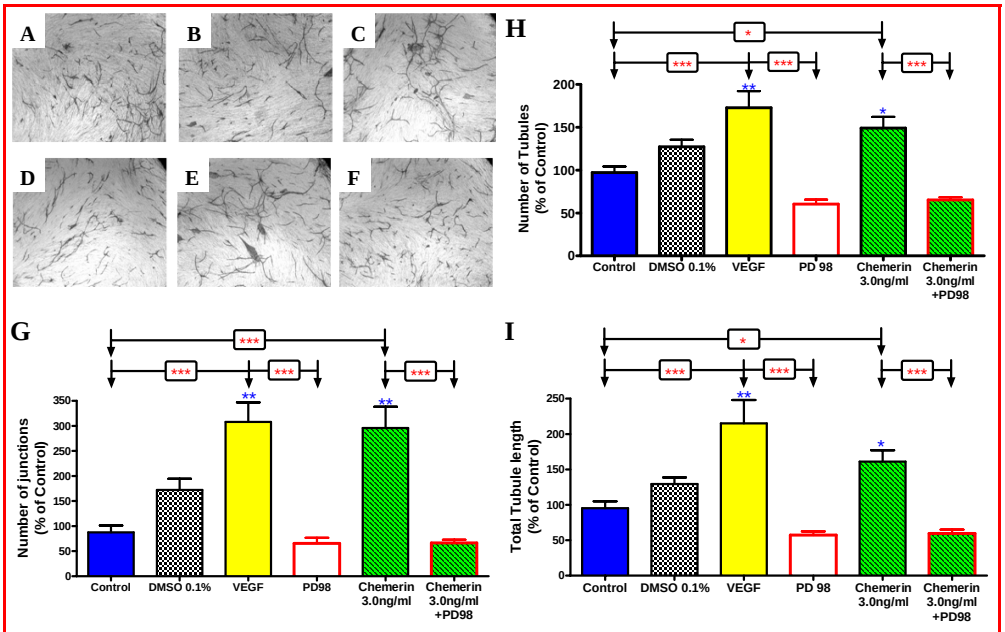

Figure 2. The effect of PD98059, a MEK1 inhibitor, on the angiogenic properties of chemerin in endothelial cells in a co-culture system. The co-culture of endothelial cells and fibroblasts was used to study the formation of new blood vessels. Recombinant human chemerin was added at 3 and 10 ng/ml respectively (in the presence or absence of 25 mM PD 98059). Cells were fixed, and stained for CD31, on day 9. **(A)** control: cell medium only; (**B**) Growth media with 0.1% DMSO **(C)** VEGF positive control (2 ng/ ml); **(D)** PD 98059 (25 mM); **(E)** recombinant human chemerin (3 ng/ml); **(F)** recombinant human chemerin (3 ng/ml) + PD 98059 (25 mM). The number of microtubule junctions (**G**), the total number of microtubules (**H**), the total microtubule length (**I**). Four images were captured per well, from four independent quadrants.